

CLAIMS

1. A method of examining thin layer structures on a surface for differences in respect of optical thickness, which method comprises:

irradiating the surface (SS) with light so that the light is internally or externally reflected at the surface,

imaging the reflected light on a first two-dimensional detector (D1),

sequentially or continuously scanning the incident angle and/or wavelength of the light over an angular and/or wavelength range,

measuring the intensities of light reflected from different parts of the surface and impinging on different parts of the detector, at at least a number of incident angles and/or wavelengths, the intensity of light reflected from each part of the surface for each angle and/or wavelength depending on the optical thickness of the thin layer structure thereon,

and determining from the detected light intensities at the different light incident angles and/or wavelengths an optical thickness image of the thin layer structures on the surface,

characterized in that part of the light reflected at said surface is detected on a second detector (D2) to determine the incident angle or wavelength of the polarized light irradiating the surface.

2. The method according to claim 1, characterized in that said part of the light for determining the incident angle or wavelength is focused on said second detector (D2) as a point or line, the position of said point or line on the detector being related to the angle or wavelength of the incident light.

3. The method according to claim 1 or 2, characterized in that the angle of the incident light is scanned.

4. The method according to claim 1 or 2, characterized in that the wavelength of the incident light is scanned.

5. The method according to any one of claims 1 to 4, characterized in that said first and second detectors (D1, D2) are different parts of a single photodetector matrix array.

6. The method according to any one of claims 1 to 5, characterized in that said light is non-coherent.

7. The method according to any one of claims 1 to 5, characterized in that said light is coherent.

8. The method according to any one of claims 1 to 7, characterized in that said light is totally internally reflected from said surface.

9. The method according to claim 8, characterized in that said light is coupled to said surface (SS) via a prism (Pr) or grating.

10. The method according to claim 8 or 9, characterized in that said determination of reflection versus angle and/or wavelength is based on evanescent wave sensing.

11. The method according to claim 10, characterized in that said determination of reflection versus angle and/or wavelength is based on surface plasmon resonance, Brewster angle, ellipsometry, critical angle or frustrated total reflection waveguide resonance.

12. The method according to any one of claims 1 to 7, characterized in that said light is externally reflected from said surface.

13. The method according to claim 12, characterized in that said determination of reflection versus angle and/or wavelength is based on external Brewster angle or external ellipsometry.

14. The method according to any one of claims 1 to 13, characterized in that said surface (SS) is a chemical or biochemical sensor surface having a number of subzones, each subzone supporting a reactant capable of interacting with a specific analyte species, and that said surface is contacted with a sample suspected of containing one or more of said specific analyte species to determine their presence in the sample.

15. The method according to any one of claims 1 to 14, characterized in that said light used for determining the angle and/or wavelength of the incident light is reflected at a part of said surface that does not exhibit said thin layer structures, especially at a part of said surface that is not contacted by said sample.

16. The method according to any one of claims 1 to 15, characterized in that it comprises determining, based on said thickness images on said surface, at least one of the corresponding surface concentration, surface concentration change, surface refractive index, surface refractive index change, surface thickness and surface thickness change.

17. An optical apparatus for examining thin layer structures on a surface for differences in respect of optical thickness, comprising:

a sensor unit having a sensing surface (SS) with a number of zones capable of exhibiting thin layer structures of varying optical thickness, particularly as the result of contact with a sample,

a light source (LS) for emitting a beam of light,

optical means (Pr) for coupling said light beam to said sensor unit,

first photodetector means (D1),

means (SO) for imaging light reflected from said different parts of the sensor surface onto said first photodetector means (D1) for detecting the intensities of the reflected light,

means (SM1, SM2) for sequentially or continuously scanning said light incident at said sensor surface over a range of incident angles and/or wavelengths (M),

means for determining each angle and/or wavelength of light impinging on said sensing surface,

evaluation means for determining from the relationship between detected intensity of the reflected light and incident light angle and/or wavelength, the optical thickness of each zone of said sensor surface to thereby produce a morphometric image of the optical thickness of the sensor surface,

characterized in that the apparatus comprises second photodetector means (D2), and that said means for determining said light angles and/or wavelengths comprise means (SO, CL1, CL2) for focusing a part of said monochromatic light reflected at the sensing surface onto the second photodetector means (D2), wherein each position of said focused light on said second photodetector means is related to a specific angle and/or wavelength of the light incident at the sensing surface (SS).

18. The apparatus according to claim 17, characterized in that said imaging means comprise a bifocal lens system comprising a first part (SO) having its real image plane at the plane of said first photodetector means (D1), and a second part (SO, CL1, CL2) having its back focal plane at the plane of said second photodetector means (D2), so that at the back focal plane the spatial reflectance is transformed to the spatial reflectance angle.

19. The apparatus according to claim 17 or 18, characterized in that the apparatus includes a wavelength dispersive element, so that at the back focal plane the spatial reflectance is transformed to the spatially reflected wavelength.

20. The apparatus according to claim 17, 18 or 19, characterized in that said first and second photodetector means (D1, D2) are different parts of a single two-dimensional matrix array of photodetector elements.

21. The apparatus according to claim 20, characterized in that said photodetector matrix array comprise a first main detector area (D1) positioned at the image plane

of the bifocal lens system, and a second detector area (D2) positioned at the back focal plane of the bifocal lens system, so that at said first main detector area each detector element via the real image of the sensor surface corresponds to a coordinate of the sensor surface, and at said second detector area each detector element via the position of the light corresponds to the angle of incidence.

22. The apparatus according to claim 20, characterized in that said photodetector matrix array comprises a first main detector area (D1) positioned at the image plane of the bifocal lens system, and a second detector area (D2) positioned at the back focal plane of the bifocal lens system, so that at said first main detector area each detector element via the real image of the sensor surface corresponds to a coordinate of the sensor surface, and at said second detector area each detector element via the position of the light corresponds to the wavelength.

23. The apparatus according to any one of claims 17 to 22, characterized in that said scanning means comprise beam deflecting means (SM1, SM2) to produce an angle-scanned collimated illumination of the sensor surface with a substantially stationary illuminated sensor surface area and area position for a given angular interval, at which sensor area each surface point momentarily is illuminated by light rays of identical angle of incidence and wavelength.

24. The apparatus according to any one of claims 17 to 23, characterized in that said optical coupling means is either a prism (Pr) or lens having at least one plane side, or a grating.

25. The apparatus according to any one of claims 17 to 24, characterized in that said evaluation means comprise an evaluation unit for determining the angle, and/or the wavelength, for minimum reflectance of p-polarized light, and/or the relative reflectance and

phase of the p- and s-polarized electric field components of the light for each of the specific multi-zones.

26. The apparatus according to any one of claims 17 to 25, characterized in that said evaluation means comprise an evaluation unit for calculating and presenting the surface structures for each of the specific multi-zones of the sensing surface.

27. The apparatus according to any one of claims 17 to 26, characterized in that said sensing surface (SS) supports reactants capable of binding interaction with species in a sample, particularly biomolecules, to produce said thin surface structures to be examined.

28. The apparatus according to any one of claims 17 to 27, characterized in that the apparatus comprises a sample solution container in contact with the sensor unit to expose the sensing surface to sample solution.

29. The apparatus according to any one of claims 17 to 28, characterized in that said angular scanning means comprise two inter-related oscillating mirrors (SM1, SM2).

30. The apparatus according to any one of claims 17 to 28, characterized in that said angular scanning means comprise two mirrors in a fixed configuration which is oscillated or rotated.

31. The apparatus according to any one of claims 17 to 28, characterized in that said angular scanning means comprise an oscillating plane mirror (SM1) and a concave mirror (Mc).

32. The apparatus according to any one of claims 17 to 28, characterized in that said angular scanning means comprise a translationally moving plane mirror in combination with a generally concave cylindrical fixed mirror.

33. The apparatus according to any one of claims 17 to 28, characterized in that said angular scanning means comprise a pivotally moving plane mirror in combination with said optical coupling means.

34. The apparatus according to any one of claims 17 to 28, characterized in that said angular scanning means comprise a rotating polygon plane mirror in combination with said optical coupling means.

35. The apparatus according to any one of claims 17 to 28, characterized in that said angular scanning means comprise two telecentric angular scanners including an oscillating plane mirror and a first focusing optical component, which creates a scanned focal line, said focal line-surface coinciding with the focal line-surface of a second focusing optical component, thus creating a collimated beam of scanned angle of incidence.

36. The apparatus according to claim 35, characterized in that said first and second focusing optical components are convex cylindrical lenses.

37. The apparatus according to claim 35, characterized in that said first focusing component is a convex cylindrical lens, and that said second virtually focusing component is a virtually focusing concave cylindrical lens.

38. The apparatus according to claim 35, characterized in that said first focusing component is a virtually focusing component in the form of a diverging (negative) lens, and the second focusing component is a convex cylindrical lens.

39. The apparatus according to claim 35, characterized in that said angular scanning means comprise a pivotally moving illumination system.

40. The apparatus according to any one of claims 18 to 39, characterized in that said first part of the bifocal lens system is a spherical lens system (SO), and that said second part of the bifocal lens system is said spherical lens system (SO) combined with a cylindrical lens system (CL1, CL2).

41. The apparatus according to any one of claims 17 to 40, characterized in that said light source (LS) emits substantially monochromatic and non-coherent light.

42. The apparatus according to claim 41, characterized in that said light source is a light emitting diode in combination with an interference filter.

43. The apparatus according to any one of claims 17 to 40, characterized in that said light source (LS) emits substantially monochromatic and coherent light.

44. The apparatus according to claim 43, characterized in that said light source (LS) is a semiconductor diode laser, a dye laser, or a gas laser.

45. The apparatus according to claim 44, characterized in that said beam deflecting means comprise a holographic beam deflector.

46. The apparatus according to any one of claims 23 to 44, characterized in that said beam deflecting means comprise an acousto-optical deflector.

47. The apparatus according to any one of claims 17 to 46, characterized in that said light source (LS) emits a continuous light spectrum, and is combined with a scanning monochromator for sequentially providing more than one wavelength.

48. The apparatus according to any one of the claims 17 to 47, characterized in that the plane of said first and second photodetector means (D1, D2) is inclined in relation to the

plane of reflection, so that the focus plane of the real image of the sensor surface coincides with the photodetector plane.

49. The apparatus according to any one of claims 17 to 48, characterized in that the apparatus comprises a wedge-shaped transparent body that corrects for defocusing refraction at an inclined observing view and within the light coupling element for the range of angle of incidence.

50. The apparatus according to any one of claims 17 to 49, characterized in that said apparatus is based on determining total internal reflection versus angle and/or wavelength of incidence.

51. The apparatus according to claim 50, characterized in that said internal reflection versus angle and/or wavelength of incidence determination is based upon surface plasmon resonance, Brewster angle, ellipsometry, critical angle, or frustrated total reflection waveguide resonance.

52. The apparatus according to any one of claims 28 to 51, characterized in that said sample solution is contained in a flow cell.

53. The apparatus according to any one of claims 17 to 52, characterized in that said sensor unit is designed to be exchangeable in the apparatus.

54. The apparatus according to any one of claims 17 to 53, characterized in that said sensor unit is an integral part of said optical coupling means, especially a prism (Pr) or grating.

55. The apparatus according to any one of claims 17 to 54, characterized in that said sensor unit comprises a first part covered by said sensing surface zones, and a second

part providing a constant reflectance during the scan of at least one of angle and wavelength of incidence.

56. The apparatus according to any one of claims 17 to 55, characterized in that the apparatus comprises an exchangeable resilient opto-coupling member designed to interface both the sensor unit and said optical coupling means.

57. The apparatus according to any one of claims 17 to 56, characterized in that said evaluation means provides for image processing where a detected non-coherent light reflectance pattern for the sensor surface is correlated to both the angle of incidence and time to create a four-dimensional real-time refractometric representation of the lateral optical thickness distribution on the sensing surface.

58. The apparatus according to any one of claims 17 to 56, characterized in that said evaluation means provides for image processing where a detected coherent light reflectance pattern and interference pattern for the sensor surface is correlated to both the angle of incidence and time to create a four-dimensional real-time refractometric representation of the lateral optical thickness distribution on the sensing surface.

59. The apparatus according to any one of claims 17 to 57, characterized in that said evaluation means provides for image processing where the detected non-coherent light reflectance pattern for the sensing surface is correlated to both the angle of incidence and/or wavelength and time to create a five-dimensional real-time refractometric representation of the lateral optical thickness distribution as a function of penetration depth of an evanescent wave, created in said total internal reflection, on the sensing surface.

60. The apparatus according to any one of claims 17 to 56 and 58, characterized in that said evaluation means provides for image processing where a detected coherent light reflectance pattern and interference pattern for the sensor surface is correlated to

both the angle of incidence and/or wavelength and time to create a five-dimensional real-time refractometric representation of the lateral optical thickness distribution as a function of penetration depth of the evanescent wave, created in said total internal reflection, on the sensing surface.

61. The apparatus according to any one of claims 51 to 60, characterized in that said sample solution container is a wall-jet flow cell, in which a jet flow is directed orthogonally against said sensing surface.

62. The apparatus according to any one of claims 21 to 60, characterized in that said photodetector matrix array comprises a first part for imaging the sensing surface, a second part for determining the angle of the incident light, and a third part for determining the wavelength of the incident light.

63. A method of calibrating an optical apparatus according to any one of claims 1 to 62, characterized in that the method comprises:

providing a specific calibration sensor unit, the sensing surface of which contains laterally distributed zones, each zone having a dielectric film of known refractive index,

providing a sensor unit as defined in any one of claims 17, 27 and 53 to 55 in contact with sample solution, the sensing surface of which may contain laterally distributed zones, each zone having a known fixed refractive index,

storing in a memory the electric signals from the photodetector elements corresponding to the known refractive index-zones within the real image produced, and the corresponding determined angle of incidence and/or wavelength, so that a known relationship is obtained between the angle or wavelength for resonance as measured through reflectance minimum or maximum, or change in polarization state, and the refractive index of the dielectric film.

64. The method according to any one of claims 1 to 16, characterized in that said determination of optical thickness images comprises:

- a) defining pixels, or clusters of pixels, on said first detector, each pixel or cluster of pixels corresponding to a respective one of a number of different zones on said surface,
- b) normalizing and storing reflectance data obtained from the different zones without said thin layer structures so that the data are identical for all incident light angles/wavelengths,
- c) storing, during a suitable number of angular and/or wavelength scans over a predetermined period of time, in a memory the raw-data images detected by said pixel elements corresponding to said zones,
- d) storing, simultaneously, in a memory angular data and/or wavelength data detected by a pixel row or rows of said second detector corresponding to a constant reflectance zone on said surface, and time,
- e) calculating the monitored angle of incidence and/or wavelength as a function of time,
- f) storing in a memory a raw data image based on the calculated angle, time and/or wavelength,
- g) calculating a normalized image for each stored raw-data by use of the image-normalization data in step b),
- h) storing the calculated angle, time and/or wavelength for the normalized image in a memory,
- i) assembling for each zone in said normalized image memory, the corresponding data for normalized reflectance/angle/wavelength/time into a reflectance curve versus angle, each angle corresponding to a specific time within the scan, and/or reflectance curve versus wavelength, each wavelength corresponding to a specific time within the scan,
- j) calculating for each zone in said memory, the angle and/or wavelength at the reflectance minimum, maximum and/or centroid for the reflectance curve, and a medium time for each scan,

k) calculating for each zone in said memory, the angular shift, and/or wavelength shift versus a medium time for the scans,

l) calculating for each zone, the change in surface concentration, refractive index or thickness from the angular shift, and/or wavelength shift, and

m) presenting the simultaneous internal and/or relative changes in surface concentration, refractive index or thickness for selected zones as graphs or tables.

65. The method according to claim 64, characterized in that said normalization comprises:

a) defining pixels, or clusters of pixels, on said first detector matrix, each pixel or cluster of pixels corresponding to a respective one of a number of different zones on said surface,

b) defining from said zones/pixels or pixel clusters zones/pixel clusters to be used as reference zones,

c) calibrating the angle and/or wavelength measurement by calculating the relation between the position of a detected focused intensity curve and the known angle of incidence and/or wavelength,

d) storing, during a suitable number of angular and/or wavelength scans, in a memory the raw-data images detected by said pixel or pixel clusters corresponding to each zone,

e) storing, during a predetermined number of angular and/or wavelength scans, as a function of time in a second computer memory the angular data and/or wavelength data detected by said pixel row or rows corresponding to the constant reflectance zone,

f) calculating the monitored angle of incidence and/or wavelength as a function of time,

g) storing in a memory the related calculated angle and/or wavelength, time, and raw data image,

h) contacting said surface with a medium having a refractive index enabling a total internal reflectance at all said zones,

i) calculating a reflectance normalization data matrix for each raw-data image stored in said raw-data image memory, and

j) storing in a memory the calculated angle, and/or wavelength, time, and image-normalization data.

66. The method according to claim 64 or 65, characterized in that the method comprises:

selecting said clusters of pixels on said detector matrix so that each cluster corresponds to the position of a respective zone on said surface, and that at least one pixel row corresponds to the position of a constant reflectance zone on said surface, and

assigning a specific center-pixel to each of the imaged zones and a cluster of neighbor-pixels around this center-pixel as a function of at least one of the size of the image of the sensor-zone, the geometry of the image of the zone, the size of the pixels of the detector matrix, the detected difference in reflectance for neighbor-pixels, the difference in electronic light-sensitivity and noise for different numbers of pixels and geometry of the pixel pattern within a cluster, and the integration time of the electronic average-response of the pixels.

67. The method according to claim 66, characterized in that the assignment of the specific center-pixels corresponding to each of the imaged surface zones and to the constant reflectance zone are performed manually, and that the method further comprises:

defining a zone pattern to thereby initiate the automatic generation of a matching default center-pixel pattern, and a position-matching and locking of this generated default pixel pattern to the image of the respective zones, and

visualizing the pixel pattern and the image of the surface zone on a screen during the scanning of at least one of the angle and wavelength of incidence.

68. The method according to claim 66 or 67, characterized in that the assignment of the specific center-pixel corresponding to each imaged surface zone, and of the

specific pixel row or rows to the constant reflectance zone are performed automatically and that the method further comprises:

defining a surface zone pattern to thereby initiate the automatic generation of a matching default center-pixel pattern,

performing an image analysis to determine the positions and borders of the surface zones within the image,

position-matching and locking the generated default pixel pattern to the determined image of the surface zones, and

visualizing the pixel pattern and the image of the surface zone on a screen during the scanning of at least one of the angle and wavelength of incidence.

69. The apparatus according to any one of claims 17 to 68, characterized in that the apparatus comprises computer means.

70. The apparatus according to claim 69, characterized in that said computer means are designed to perform the method according to any one of claims 64 to 68.

71. The apparatus according to any one of claims 17 to 62, 69 and 70, characterized in that the apparatus comprises optical means (P) placed between the light source (LS) and the detector (D) for polarizing said light.

72. The apparatus according to any one of claims 17 to 62, 69 and 70, characterized in that said light source (LS) emits polarized light.

73. The method according to any one of claims 1 to 16, characterized in that said light for monitoring angle and/or wavelength passes through at least one aperture in at least one obscuration.

74. The apparatus according to any one of claims 17 to 72, characterized in that said apparatus comprises at least one aperture in at least one obscuration positioned in at least one part of the beam scanned in at least one of the angles or wavelengths of incidence, for determining the cross-section geometry of the light beam used for monitoring angle and/or wavelength.